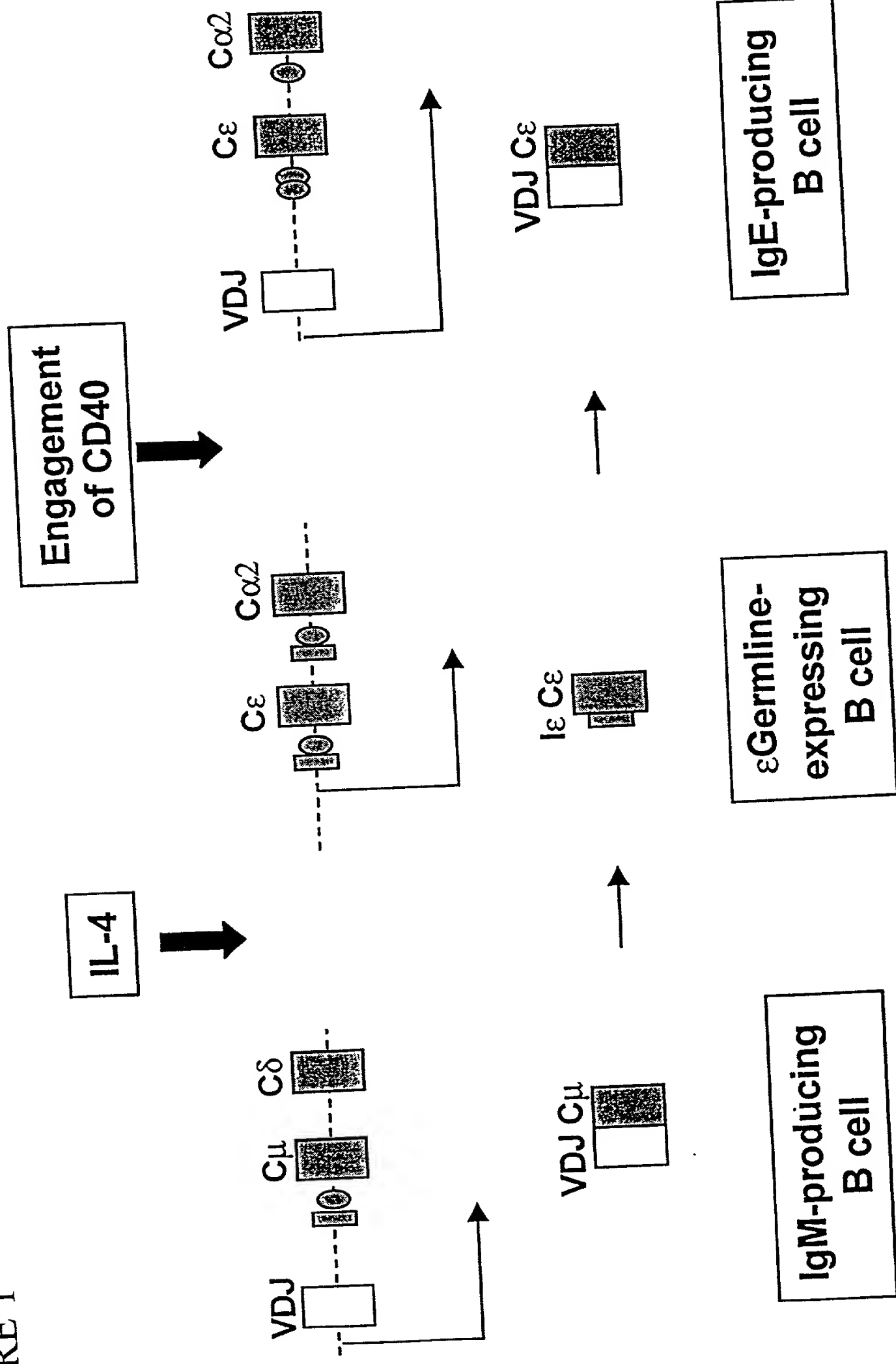


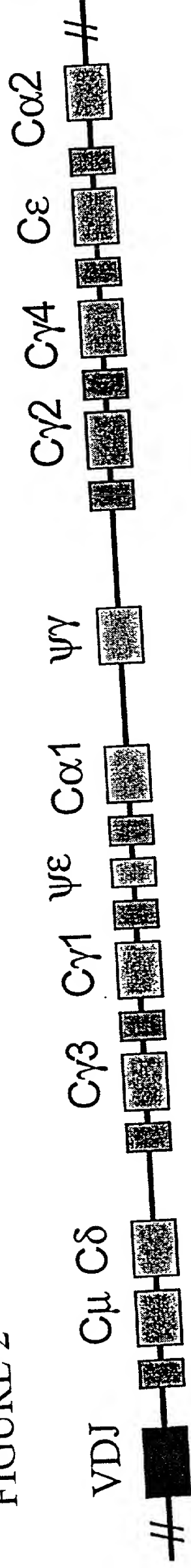
Germline Transcription and IgE Switching

FIGURE 1

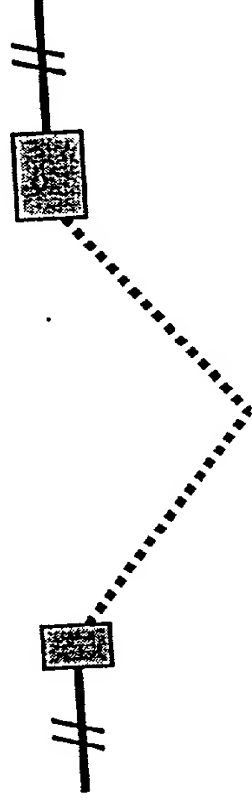


Chromosome 14 Human Heavy Chain Gene Map

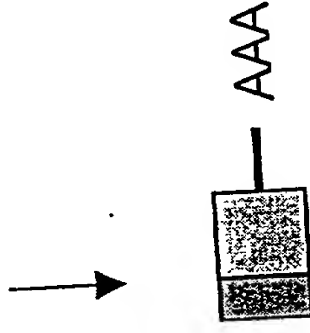
FIGURE 2



Primary Germline Transcript



RNA Processing



■ = | exon

■ = Constant exons

Spliced Germline Transcript

Sequences of RPA Probes for Human Immunoglobulin
Germline Transcripts

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGGGCGGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCCGTGAGGGTGGACCTGCCATGAGGGCCTGCACGCCGGAG
GCCGCCCACTCAGCACTGCGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCACGTGAAGCACTACACGAATCCCA
GCCAGGATGTGACTGTGCCCTGCCCAGT
TCCCCCACCTCCCCCATGCTGCCACCCCCGACTGTCGCTGCACCGACCGGCCC

Germline Ig Epsilon Probe

GGCTCCACTGCCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCATCCACAGGCACCAAATGGACGACCCGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCTTG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCGTG

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGCCCCAGGTG
AACGGAGGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCC
TGGCACCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACCTCAGGCGCCCTGA
CCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAAAGTTGAGCC
CAAATCTTGTGACAAAACCTCACACATGCCCACCG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACACCAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGCCCCAGGTGAACGGAGGGGCTTGTCCAGGCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGGTGACGGTGTCGTG
GAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCG
TGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGT
AGATCACAAGCCCAGCAACACCAAGGTG
GACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCAC
CACCTGTGGCAGGACCGTCA

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGGCCCATCGGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGA
CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACACCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAGAGTTGAGCT
CAAAACCCCACTTGGTGACACAACCTCACACATGCCACGGTGCCCAGAGCCC
AAATCTTGAGACACACCTCCCCCGTGCC
CACGGTGCCC

[illegible]

GGCCAGCACCA.CATGGAAGCCCAAGCGGAGCCAGCACGGGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCCACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGGCTTCCAAGCCAACAGGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGGCAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGGAGAGGCCCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGGGCCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCCTGGTCAAGGA
CTACTTCCCCGAACCGGTGACGGTGTCGTGGA ACTCAGGCGCCCTGACCAGE
GGCGTGACACCTTCCCGGCTGTCCTAC
AGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG
CTTGGGCACGAAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAA
TATGGTCCCCCGTC

Sequences of RPA Probes for Human Immunoglobulin Germline Transcripts

Germline Ig Alpha-1 Probe

GGCCTGGGCGCGGCCAGGGCTCCCTCCCCACAGCAGGCTCTTGGCAGGCAG
CCAGACGCCCCGTGAGGGTGGACCTGCCA
TGAGGGCCTGCACGCCGGAGGCCGCCCACTCAGCACTGCGGGCCCTCCAGCA
GCCTGACCAGCATCCCCGACCAGCCCCA
AGGTCTTCCCGCTGAGCCTCTGCAGCACCCAGCCAGATGGGAACGTGGTCAT
CGCCTGCCTGGTCCAGGGCTTCTTCCCC
CAGGAGCCACTCAGTGTGACCTGGAGCGAAAGCGGACAGGGCGTGACCGCC
AGAAACTTCCCACCCAGCCAGGATGCCTC
CGGGGACCTGTACACCACGAGCAGCCAGCTGACCCTGCGGCCACACAGTGC
CTAGCCGGCAAGTCCGTGACATGCCAC

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGCGCCGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCCGTGAGGGTGGACCTGCCATGAGGGCTGCACGCCGGAG
GCCGCCCACTCAGCACTGCGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCTACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCAC

Germline Ig Epsilon Probe

GGCTCCACTGCCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCATCCACAGGCACCAAATGGACGACCGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCTTG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCTGTG

10250-0964360

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGCTGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGCCCCAGGTG
AACGGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAATCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTECACCAAGGGCCATCGGTCTTCCCCC
TGGCACCCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACACCAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGCCCCAGGTGAACGGAGGGGCTTGTCCAGTCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTTCCTGGTCAAGGACT
ACTTCCCCGAACCGG

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGCTGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAATCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGCCATCGGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACCTCAG

Germline Ig Gamma 4 Probe

GGCCAGCACCATGGAAGCCCAAGCGGAGCCAGCACGGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCCACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGGCTTCCAAGCCAACAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGGCAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGGAGACGGCCCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGGACCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCCTGGTCAAGGA
CTACTTCCCCGAACCGG

T02050-0964360

FIGURE 5

10250-0964360

RPA PROBES

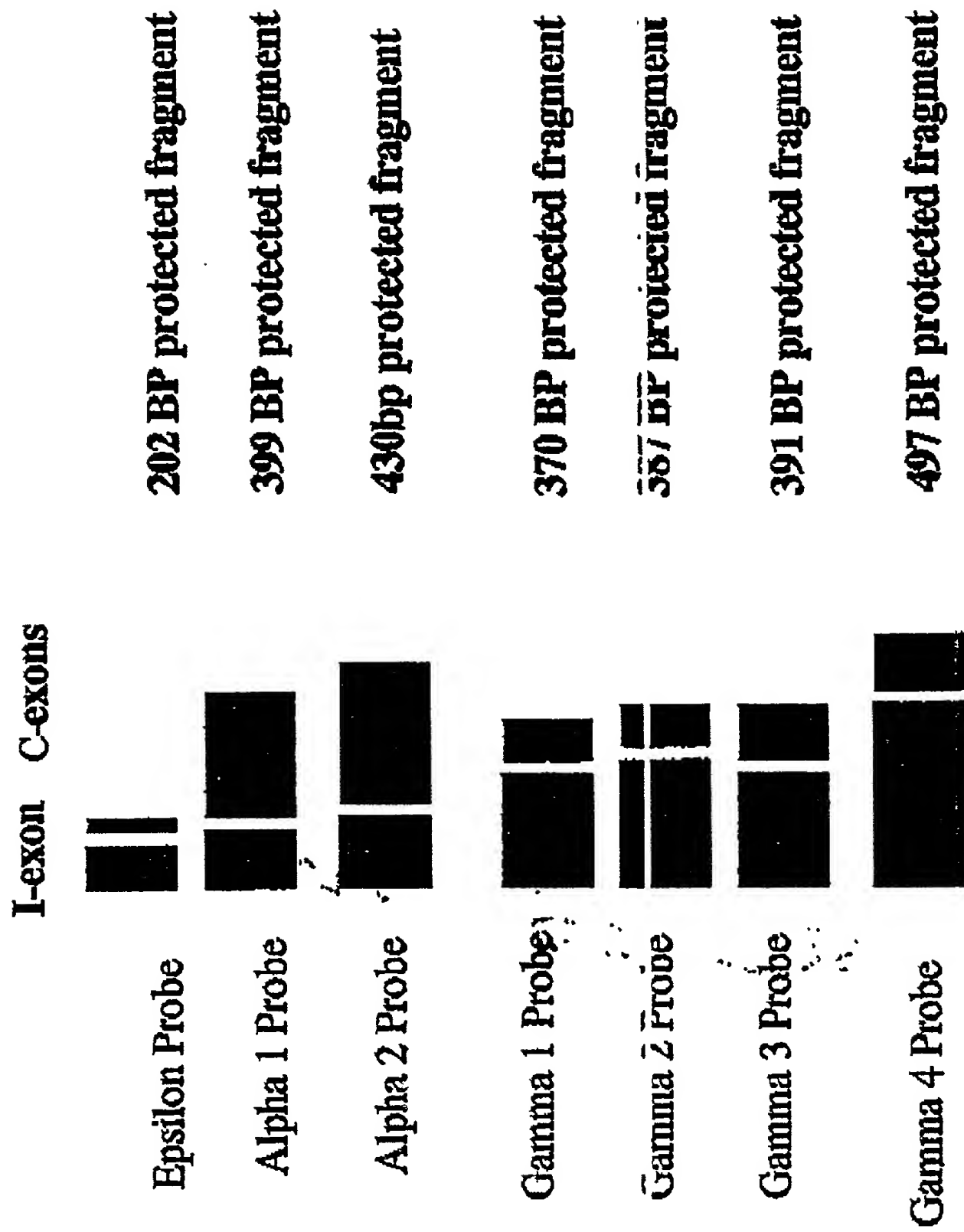
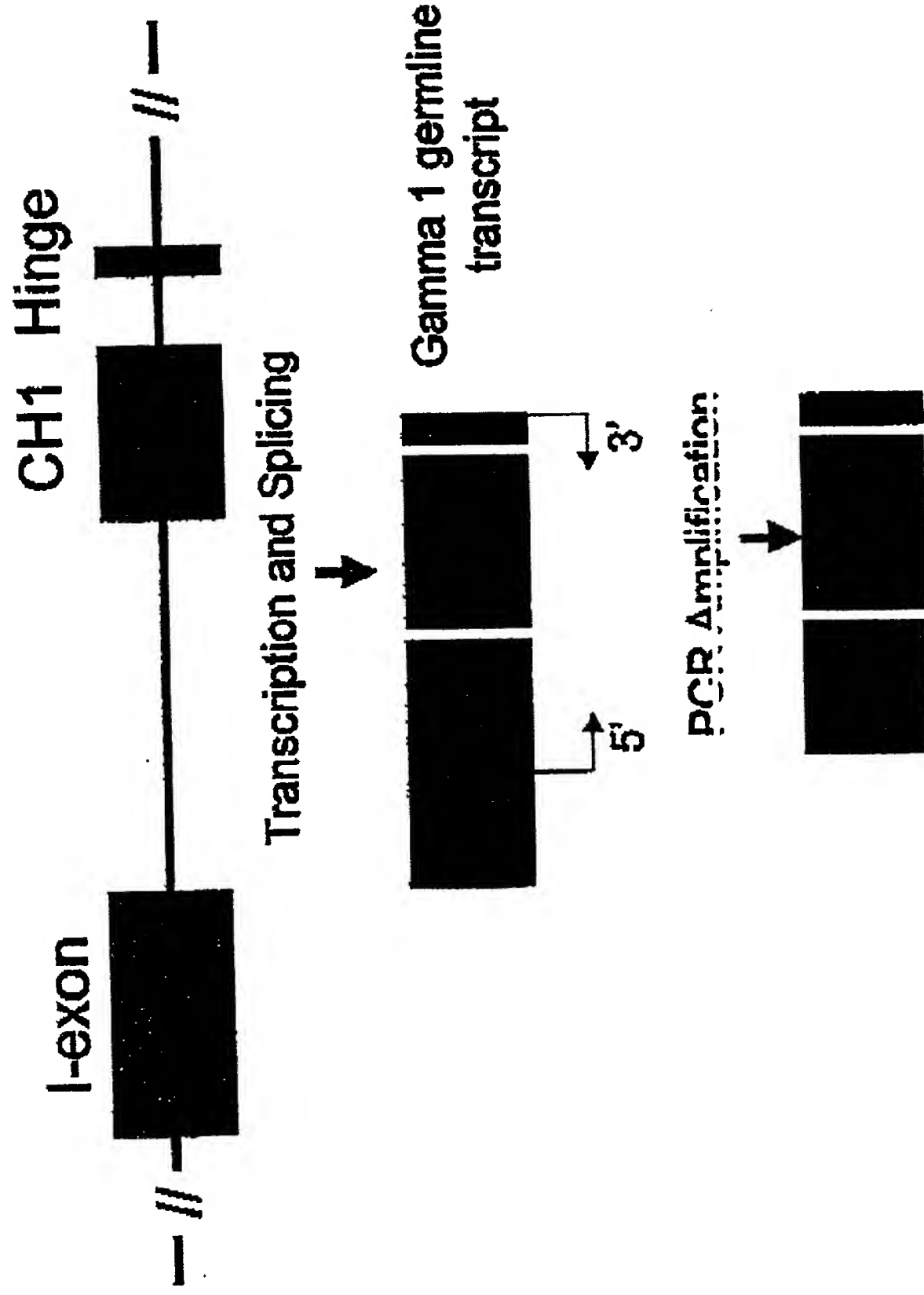


FIGURE 6

Gamma 1 Probe



The Gamma 1 5' and 3' Primers amplified a completed probe of 370 BP

RNAse Probe Protection Assay

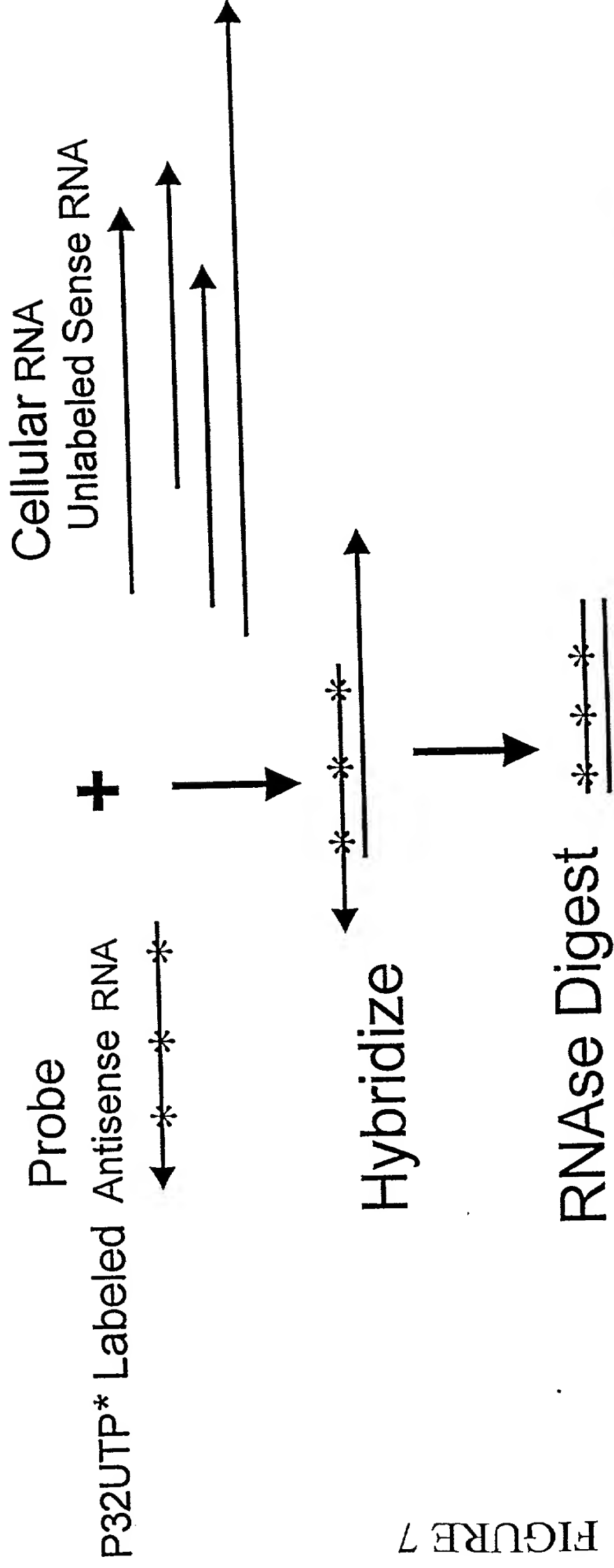
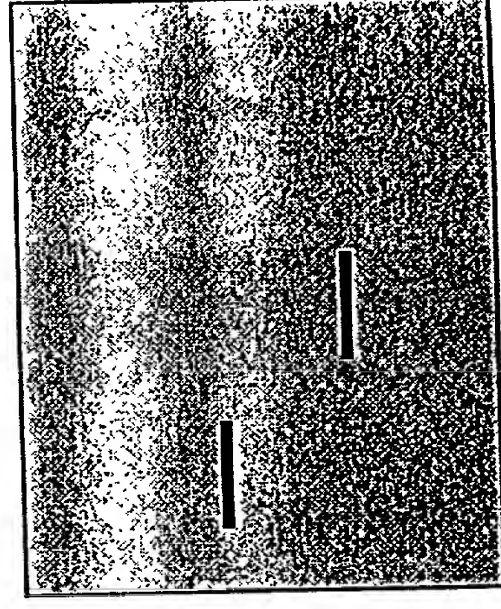


FIGURE 7

Run undigested probe vs digested protected fragment on acrylamide-Urea gel



Undigested Probe
Protected Fragment

Visualize using beta imaging equipment

FIGURE 8

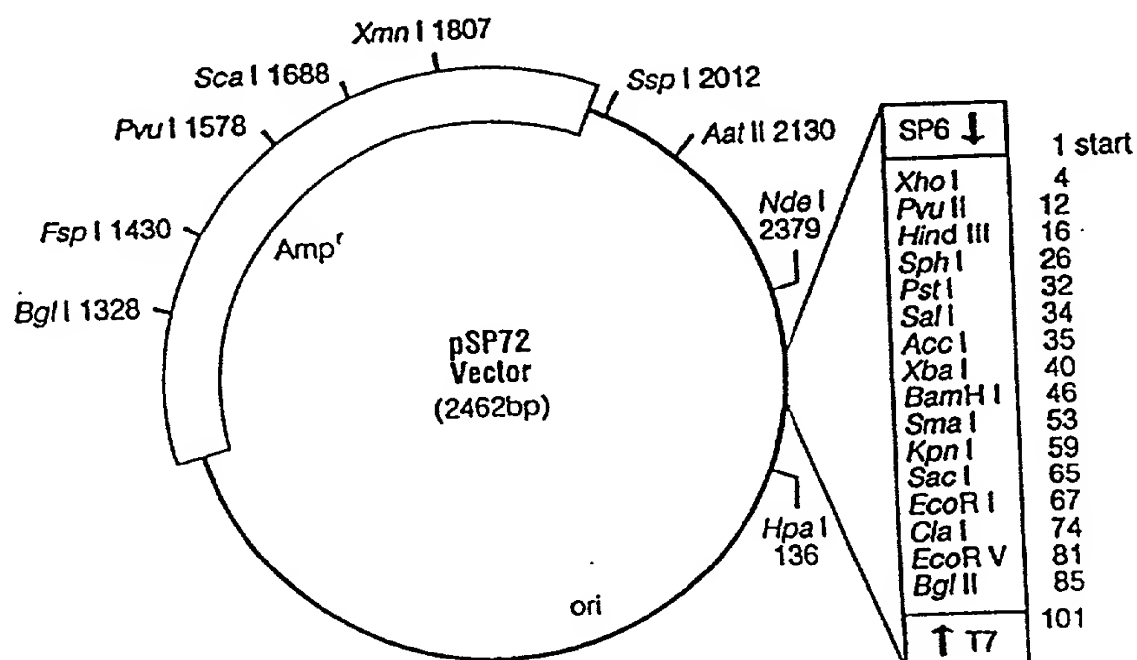


Figure 2. pSP72 Vector circle map and sequence reference points.

- Sequence reference points:
 - SP6 RNA polymerase transcription initiation site 1
 - T7 RNA polymerase transcription initiation site 101
 - SP6 RNA polymerase promoter 2446-6
 - T7 RNA polymerase promoter 96-118
 - multiple cloning sites 4-90
 - β -lactamase (Amp^r) coding region 1135-1995
- Specialized application:
 - transcription *in vitro* from dual opposed promoters (For protocol information, please request Promega's Riboprobe® *in vitro* Transcription Systems Technical Manual, #TM016.)
- The pSP72 and pSP73 Vectors are identical except for the orientation of the multiple cloning region.
- Blue/white screening for recombinants is not possible with the pSP72 Vector.

Accession Numbers for Germline Transcripts

Alpha - 1

L04541 = I Region Exon
BC005951 = Constant Region Exon

Alpha - 2

L04541 = I Region Exon
AL389978 = Constant Region Exon

Epsilon

X56797 = I Region Exon
J00222 = Constant Region Exon

Gamma - 1

AL122127 = I Region Exon
Z17370 = Constant Region Exon

Gamma - 2

U39934 = I Region Exon
J00230 = Constant Region Exon

Gamma - 3

AL122127 = I Region Exon
X16110 = Constant Region Exon

Gamma - 4

X56796 = I Region Exon
K01316 = Constant Region Exon